1978

The Columnar Junipers of Western North Dakota (An Investigation into the Cause of their Columnarity)

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THE COLUMNAR JUNIPERS OF WESTERN
NORTH DAKOTA (AN INVESTIGATION INTO
THE CAUSE OF THEIR COLUMNARITY)

by

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A thesis submitted
in partial fulfillment of the the requirements for the
degree of Master of Science, Major in
Botany-Biology, South Dakota
State University

1978

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THE CAUSE OF THEIR COLUMNARITY)

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ACKNOWLEDGEMENTS

I wish to express my appreciation to my advisor, Dr. David Holden, for his assistance and guidance in the completion of this thesis.

I would also like to thank the many other people who showed an interest and helped me with my study, especially Elmer Eide who assisted me with the gas chromatography.

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INTRODUCTION

Description of Site

In the badlands of western North Dakota there are numerous lignite beds. The area is one where the forces of erosion have cut deep gullies in the soil, exposing the various strata of sediment, including lignite, and leaving many buttes and steep hillsides in an otherwise seemingly endless grassland. Many of the exposed veins of lignite have been ignited by prairie fire or lightning, and have burned underground at a very slow rate with oxygen limiting. The overlying materials which are mostly clays, are baked into a reddish-brown material locally known as scoria. As the fire proceeds and the lignite is turned to ash, the scoria and other overburden collapse into the cavity that is left behind. This results in large cracks and holes in the overburden which extend from the burning lignite to the soil surface, a distance which has been estimated to be approximately 30 feet (U.S.F.S. Pamphlet). It is through these vent holes that the fumes from the fire escape into the atmosphere and oxygen for the combustion process is supplied. Today there are still several of these lignite veins burning.

In the summer of 1977 two of these burning lignite veins were visited. One is located in Slope County (T. 136N, R. 102W, Sec. 11, 12, 13 and 14) and the other is located in Billings County (T. 140N, R. 101W, Sec. 23). According to Staudinger (1966), the lignite vein in Slope County started burning sometime before 1880. The lignite vein in Billings County, which is in the south unit of Theodore Roosevelt National Memorial Park, was ignited by lightning in September, 1951 (Staudinger

1966).

The Columnar Junipers

The *Juniperus scopulorum* Sarg. growing in the vicinity of these burning lignite veins have taken on a columnar form. A typical *J. scopulorum* has been previously described as erect or divided into several more or less ascending trunks with the height of the tree 1 to 2 times its width. The columnar junipers, on the other hand, all have a single trunk with the height of the tree 2.5 to 3 times its width (Fassett 1944, 1945). Figure 1 shows the distribution of the columnar junipers in Slope County.

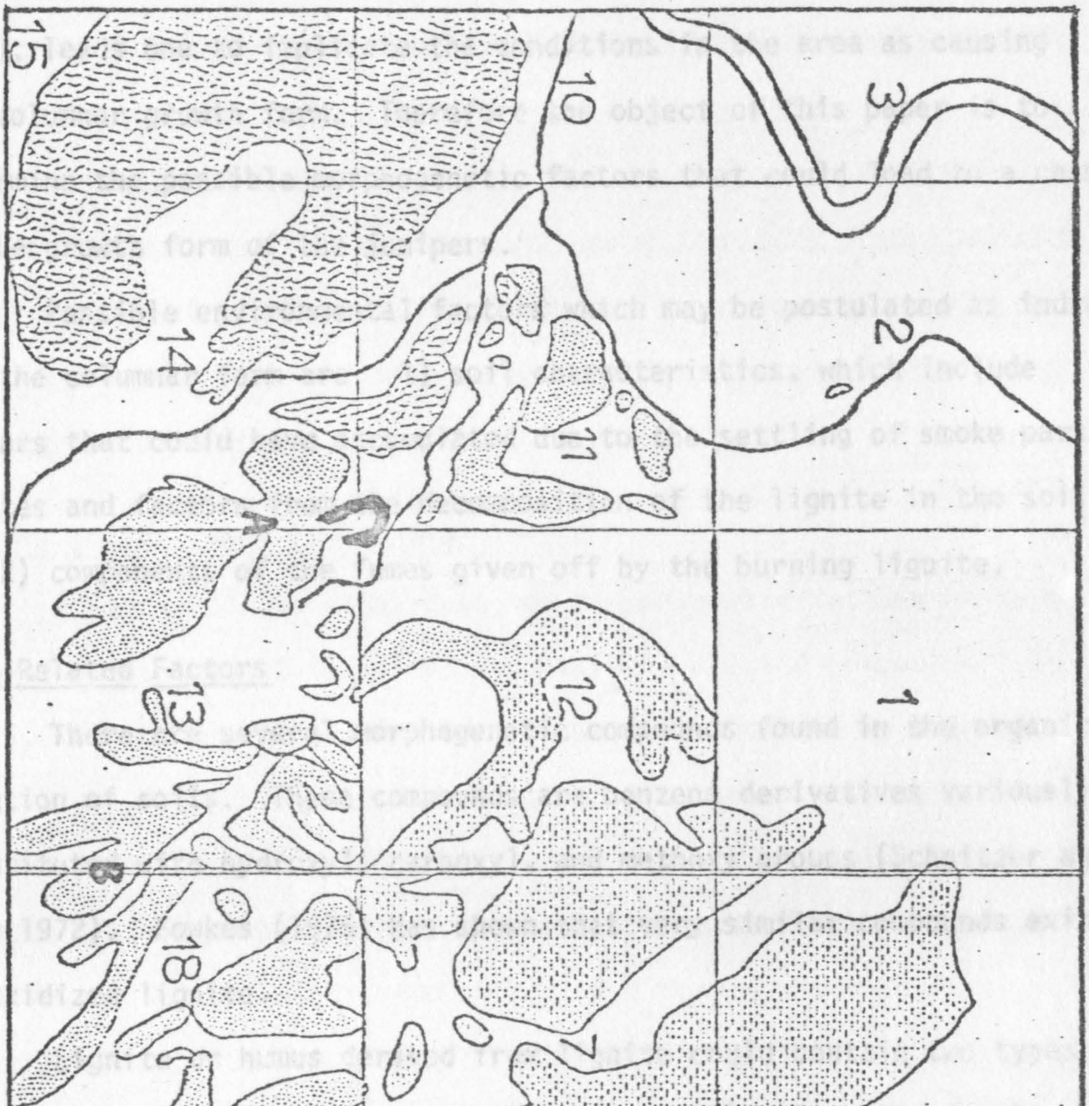
Staudinger (1966) did a chromatographic and ecological study of the columnar junipers in order to determine their proper classification. According to Staudinger, the columnar junipers had previously been considered to comprise 1) a variety of *J. scopulorum* (*J. scopulorum* var. *columnaris* Fassett); 2) an ecological variant of true *J. scopulorum*; 3) a hybrid between *J. scopulorum* and one of the other juniper species in the area; or 4) a distinct species. The evidence provided by Staudinger supported the theory that the columnar junipers are an ecologically-induced variant of true *J. scopulorum* with the phenotype requiring a certain environment for expression.

Possible Causes of Columnarity

Because of the close association between the burning litnite veins and the columnar junipers, plus the fact that the only place *J. scopulorum* has been found growing in the columnar form has been on

Figure 1. Distribution of Columnar Junipers in Slope County, North Dakota (adapted from Standinger 1966). The letters (A, B, and C) represent areas sampled for height vs. width ratio of junipers.

- Burning coal vein
- Columnar juniper
- *Juniperus scopulorum*
- *Pinus ponderosa*


15
10
3
14
2
11
A
13
12
1
B
18
6
C
7
5
8

sites where fumes of a combustion process were present (Staudinger 1966), leads one to implicate the conditions in the area as causing the columnar growth form. Therefore the object of this paper is to determine the possible morphogenetic factors that could lead to a change in the growth form of the junipers.

Possible environmental factors which may be postulated as inducing the columnar form are: 1) soil characteristics, which include factors that could have accumulated due to the settling of smoke particulates and factors from the decomposition of the lignite in the soil, and 2) components of the fumes given off by the burning lignite.

Soil Related Factors

There are several morphogenetic compounds found in the organic fraction of soils. These compounds are benzene derivatives variously substituted with hydroxyl, carboxyl, and methoxy groups (Schnitzer and Khan 1972). Fowkes (1975) has shown that very similar compounds exist in oxidized lignite.

Lignite or humus derived from lignite could contain two types of soil related morphogenetic factors (Fowkes 1975). One type is the phenylpropanoid compounds which are inhibitory in nature such as coumarin, caffeic acid, and ferulic acid (Weaver 1972). The other type, stimulatory in nature, is the humic substances such as fulvic acid (Schnitzer and Khan 1972). In an investigation by Linehan (1976), it was shown that the growth of cultured isolated tomato roots was increased by the addition of fulvic acid to the nutrient medium. It has also been shown that humic substances promoted root initiation and overall plant growth, possibly by enhancing the availability of certain nutrients or through an interaction with other growth regulators (Schnitzer and Khan 1972; Freeman and Fowkes 1968).

Since these morphogenetic factors which are normally found in soil can be derived from lignite, one would expect to find abnormally large amounts of these compounds in the soils on lignite bearing strata. Experiments were run to determine if there is a morphogenetic factor in the lignite of Slope County.

Factors in the Fumes of Burning Lignite

A search of the literature for morphogenetic factors in burning lignite fumes leads to ethylene and ethylene related compounds (Landers *et al* 1961). For many years it has been known that ethylene is a product of combustion of organic materials (Abeles 1973, Treshow 1970, Sandberg *et al* 1975, Crocker 1948). Most of the gases produced by the burning lignite are not morphogenetic. Several compounds, such as hydrogen sulfide, oxides of sulfur, and oxides of nitrogen are phytotoxic. When concentrations of these compounds get high enough they cause chlorotic or necrotic spotting of the vegetation (Treshow 1970, McCallan *et al* 1936). Most saturated hydrocarbon gases are almost completely inert as far as plants are concerned, except at extremely high concentrations (1000 ppm or higher) (Crocker 1948). It is highly improbable that such concentrations could ever be attained in the area of the burning lignite vein. Unsaturated hydrocarbon gases such as ethylene, acetylene, and propylene in addition to carbon monoxide all exhibit similar plant growth regulation properties (Crocker 1948). However, ethylene is active at much lower concentrations than any of the other gases. Crocker (1948) lists the minimum concentration (PPM) needed to elicit a tropic response in sweet pea as follows: ethylene (0.2), acetylene (250), propylene (1000), carbon monoxide (5000). He also lists data on epinasty of tomato as follows: ethylene (0.1), acetylene (50), propylene (50), carbon monoxide (500). Note that the concentration needed to promote a response with compounds other than ethylene is greater by at least a log factor of 2 to 3. Ethylene has been shown to induce leaf epinasty with as little as 1 ppb in African marigold (Crocker 1948).

The objective of this report is to focus on the evaluation of the presence and amount of ethylene in the burning lignite fumes and to search for morphogenetic factors in the lignite at the Slope County site. The former was considered to be more important and was investigated more thoroughly.

Ethylene

Ethylene has been shown to be associated with a wide variety of plant physiological processes (Abeles 1973). Some of these are highly characteristic, such as the triple response of etiolated pea seedlings (inhibition of elongation, swelling below the plumular hook, and transverse geotropism) and epinasty of tomato petioles, which has been shown to be caused by swelling of the upper cells of the basal end of the petiole (Crocker 1948). Other ethylene associated responses include induction of adventious root formation, dormancy, senescence and abcission, fruit ripening, exudation, flower induction, hook closure, and apical dominance.

There is not much literature about ethylene as it relates to tree species. There are some reports of fruit growers using ethylene releasing chemicals to initiate abscission of the fruit to facilitate mechanical harvesting (de Wilde 1971). There are also several reports on ethylene levels in the wood of stressed trees (Leopold *et al* 1972, Robitaille and Leopold 1974). It has been shown that by tying tree branches into a position which is bent from its normal position, the ethylene content of the branches increased, elongation of the branches decreased, and the radial growth of the branches increased (Brown and Leopold 1973, Leopold *et al* 1972).

It has been suggested that ethylene may serve as a natural stimulator of radial growth associated with physical stresses such as wind sway (Brown and Leopold 1973, Neel and Harris 1971). It was found that when *Pinus radiata* were stayed with guy wires so they could not sway with the wind, their stems elongated at a faster rate than their free swaying controls (Jacobs 1954).

Heck and Pires (1962) fumigated juniper and arbovitae trees for ten days with 5 ppm of ethylene. They observed a 50% abscission of the cones and branchlets of both species and about 50% kill of the terminal branches in the juniper.

MATERIALS AND METHODS

The methods and materials section is divided into an on-site investigation of the area near the burning lignite vein in Slope County and laboratory studies. The laboratory studies consisted of analysis of the fumes from simulated low oxygen burning of lignite and the extraction and study of possible plant growth regulators from lignite.

Site Evaluation

The study of the site, adjacent to the burning coal vein in Slope County, included measurement of the height and width of the columnar junipers and analysis of the fumes being emitted from the burning lignite for ethylene. The simplest method for accurately measuring ethylene concentrations in the air is by the use of gas chromatography (Abeles 1973). Ethylene can also be quantitatively measured by means of bioassays (Abeles 1973) utilizing the characteristic responses of etiolated pea seedlings (Noggle and Fritz 1976) and tomato petioles (Ross 1974).

Gas Chromatography

Samples of the fumes were collected from four different vent holes directly above the burning coal and eight other points in a randomized pattern up to 100 yards from these holes. All samples were collected in gas-tite bottles with three replications per collection point. The samples were then returned to the laboratory and analyzed by gas chromatography.

In the laboratory, 1 ml samples were injected into a Varian-aerograph series 1520 gas chromatogram equipped with a hydrogen flame ionization detector. The column was 1/8 inch by 73 1/2 inches packed with 60/80 mesh alumina and operated at 175° C. One ml samples of various concentrations of commercially obtained ethylene were also run on the chromatogram as a standard.

Ethylene Bioassays

For the bioassay, etiolated pea seedlings (*Pisum sativum* var *alaska*) were grown in moist vermiculite until they were from 1-2.5 cm tall. Two pots with ten seedlings in each were then placed at each of the twelve gas collection points described above. An additional two pots were placed approximately 500 yards from the burning lignite vein as a control. During the day the seedlings were covered with a cardboard box and at night they were uncovered. After 72 hours the seedlings were evaluated for ethylene induced responses.

Twenty-six tomatoes (*Lycopersicon esculentum* var *Sioux*) were also set out at the same time as the peas. They were grown in moist vermiculite until they were approximately 20 cm tall. The angle between the main stem and each petiole was measured and recorded. For an illustration of the petiole-stem axis angles that were utilized see Figure 10. Two plants were then placed at each of the twelve gas collection points and the control site described above.

Laboratory Studies

In addition to the above work at the site, several samples of lignite were collected in 1976 by Robert Pollock with permission from the United States Forest Service. The samples were then returned to the laboratory for further studies.

Lignite Fumes Analysis (G. C.)

The apparatus in Figure 2 was designed to simulate the burning of lignite in a low oxygen environment. Two grams of lignite were placed in the test tube and the test tube was heated to drive out all of the excess air and water vapor. The lignite was then heated until it was red-hot and the fumes were collected in a flask by water displacement. The temperature of the flame from a Bunsen burner ranges between 800° and 1000° C (Hurlbut 1956). The temperature in the test tube was found to be near 800° C since pyrex melts at 800° C and in most tests the test tube just started to melt.

The apparatus was tested before use and samples were collected after excess air and water vapor were driven off. The fumes were then analyzed on a gas chromatogram following the procedure described above.

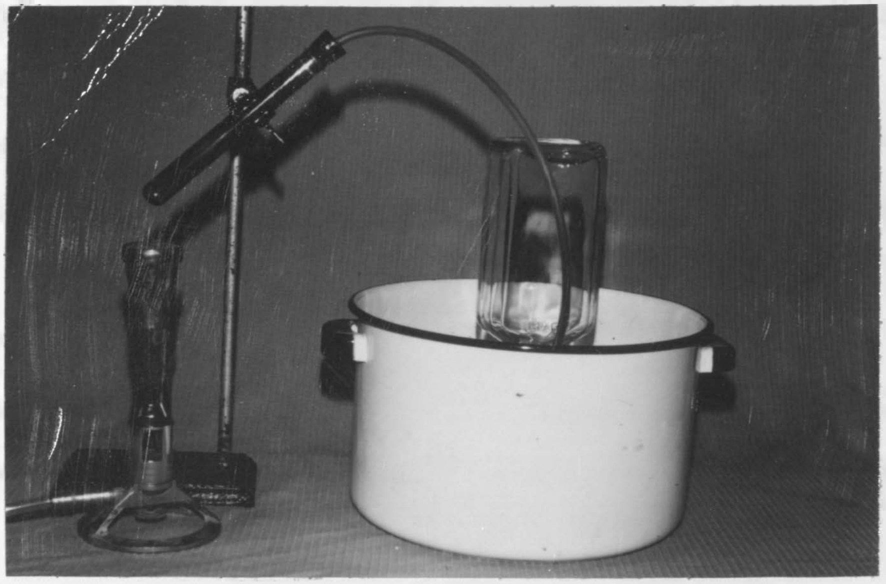
Ethylene Bioassays

Both the etiolated pea seedling and the tomato petiole bioassays were carried out in 10 liter air-tite plastic bags. The gases used as treatments were introduced into the bag with a hypodermic needle and syringe. All treatments, including the control, were carried out in triplicate.

One pot with ten pea seedlings (1-2.5 cm tall) was placed in each bag. In addition to the control there were six other treatments as follows: ethylene at 0.1 ppm, 1.0 ppm, and 10 ppm; lignite fumes at 0.01 ml/l, 0.1 ml/l, and 1 ml/l. After 72 hours the peas were removed from

Figure 2. Apparatus for burning lignite with a limited amount of oxygen. The test tube was heated to 600° - 800 ° C and the fumes were collected in the inverted bottle by water displacement.

Figure 3. Soxhlet extraction apparatus utilized for extraction of water soluble compounds from lignite. The solution in the flask is the crude lignite extract as used in this study.



the bag and the total length from the cotyledons to the plumular hook was measured to the nearest mm.

Tomatoes approximately 20 cm tall were utilized for the tomato petiole bioassay. The petiole-stem axis angles were measured and recorded. One plant was placed in each bag and the treatments were as follows: control; ethylene at 0.1 ppm, 0.5 ppm, and 1 ppm; and lignite fumes at 0.05 ml/l and 0.1 ml/l. After 24 hours the plants were removed from the bags and the petiole-stem axis angles measured again.

Lignite Extracts

Lignite from Slope County was fractionated into three main fractions based upon their solubility. The fractions, which were similar to the fractions of humic substances described by Schnitzer and Khan (1972), were: a) humic acid, which is soluble in water but is precipitated by acidification of the solution; b) fulvic acid which is soluble in water and remains in solution upon acidification; and c) humin, which is insoluble in water. There is evidence that all three fractions are of similar chemical structure, but they differ in molecular weight, functional group content, and ultimate analysis (Schnitzer and Khan 1972). Since the fulvic acid fraction is generally believed to be of lower molecular weight and is more soluble than the humic acid fraction, it has been examined by others for growth regulation properties (Forsyth 1946, Schnitzer and Khan 1972).

The Soxhlet extraction apparatus (Figure 3) was utilized for the first separation step. Thirty grams of powdered lignite was extracted with 125 ml of distilled water for 24 hours. The water insoluble residue (humin) was discarded. A portion of the aqueous extract was retained and the remainder was further separated by acidification with 1 N HCl to pH 2.5. The acidified solution was left standing for 24 hours to allow a precipitate to form. The precipitate was then suction filtered. The remaining crude extract, the acidic aqueous solution (fulvic acid), and the precipitate (humic acid) were evaporated to dryness over a steam bath. The resulting residues were then utilized in cucumber root growth tests and apical dominance tests.

Cucumber Root Growth Test

For the cucumber root growth test (Mitchell and Livingston 1968), nine ml of a test solution was added to a petri dish lined with filter paper. In all there were 16 test solutions which included the following: distilled water control; crude lignite extract at 10,000 ppm, 1,000 ppm, 100 ppm, 10 ppm, and 1 ppm; humic acid at 10,000 ppm, 1,000 ppm, 100 ppm, 10 ppm, and 1 ppm; and fulvic acid at 10,000 ppm, 1,000 ppm, 100 ppm, 10 ppm, and 1 ppm. Fifteen cucumber seeds (*Cucumis sativus* var. *marketeer*) were then placed in each of the 16 petri dishes. After 120 hours the length of the primary roots, of the ten longest roots per petri dish, were measured to the nearest mm.

Apical Dominance Test

For the apical dominance test (Machlis and Torrey 1956), 24 etiolated pea seedlings were grown in moist vermiculite for five days. Twenty of these were then decapitated just below the lowest pair of leaves. The twenty plants were divided into five treatments. The treatments, which consisted of applying a lanolin paste containing growth regulating chemicals to the decapitated stems, were as follows: 1) lanolin control; 2) lanolin containing 1000 ppm indole acetic acid (IAA); 3) lanolin containing 1000 ppm gibberellic acid (GA); 4) lanolin containing 1000 ppm triiodo-benzoic acid (TIBA), and 5) lanolin containing 100,000 ppm crude lignite extract. The lanolin pastes were renewed every 48 hours. After 14 days of treatment the length of the axillary buds, which developed in the axils of the cotyledons, were measured to the nearest mm.

Twenty-four bean seedlings (*Phaseolus vulgaris* var. *bountiful*) were treated in a similar manner as the peas except for two differences. The first of these is that the beans were grown in the light and the second is the treatments lasted for 18 days instead of 14 days.

RESULTS

Juniper Evaluation

Figures 4 and 5 depict the various forms of *J. scopulorum* observed in the North Dakota badlands. The columnar junipers (Figure 4) have only a single main trunk and the height to width ratio was found to be 3.22 for the 76 trees measured (Figure 1, Area A). The branches of the columnar junipers are more finely divided than the true *J. scopulorum* giving the columnars a dense outer layer of leaves with almost no living leaves on the inner portion of the tree. The true *J. scopulorum* (Figure 5A) usually has several erect trunks originating from near the ground. Staudinger (1966) reported a study of *J. scopulorum* in the North Dakota Badlands where the average ratio of height to width was 1.27.

In addition to the true form and the columnar form, there are several intermediate forms of *J. scopulorum* found in the North Dakota Badlands. Intermingled among the columnar junipers there are trees that have the rounded form of the true *J. scopulorum* but they also have the dense outer leaf covering characteristic to the columnar form (similar to Figure 5C). At the Billings County site there are junipers that have the rounded form in the lower portion of the tree and a columnar form in the upper portion (Figure 5B).

It was also found that as the distance from the burning lignite vein is increased, the height to width ratio of the columnar junipers decreases (Figure 5C). The height to width ratio was found to be 2.48 and 1.73 at distances of 1 and 2 miles respectively (Figure 1, Areas B

Figure 4. The columnar junipers of Western North Dakota.

A. A stand of columnar junipers near the burning lignite vein in Slope County. Note the junipers with the rounded form intermingled among the columnar junipers. It is believed that these rounded junipers had their basic growth form established prior to the start of the fire.

B. A selected individual showing the columnar form. Note the dense outer layer of leaves and the single main stem.


A


B

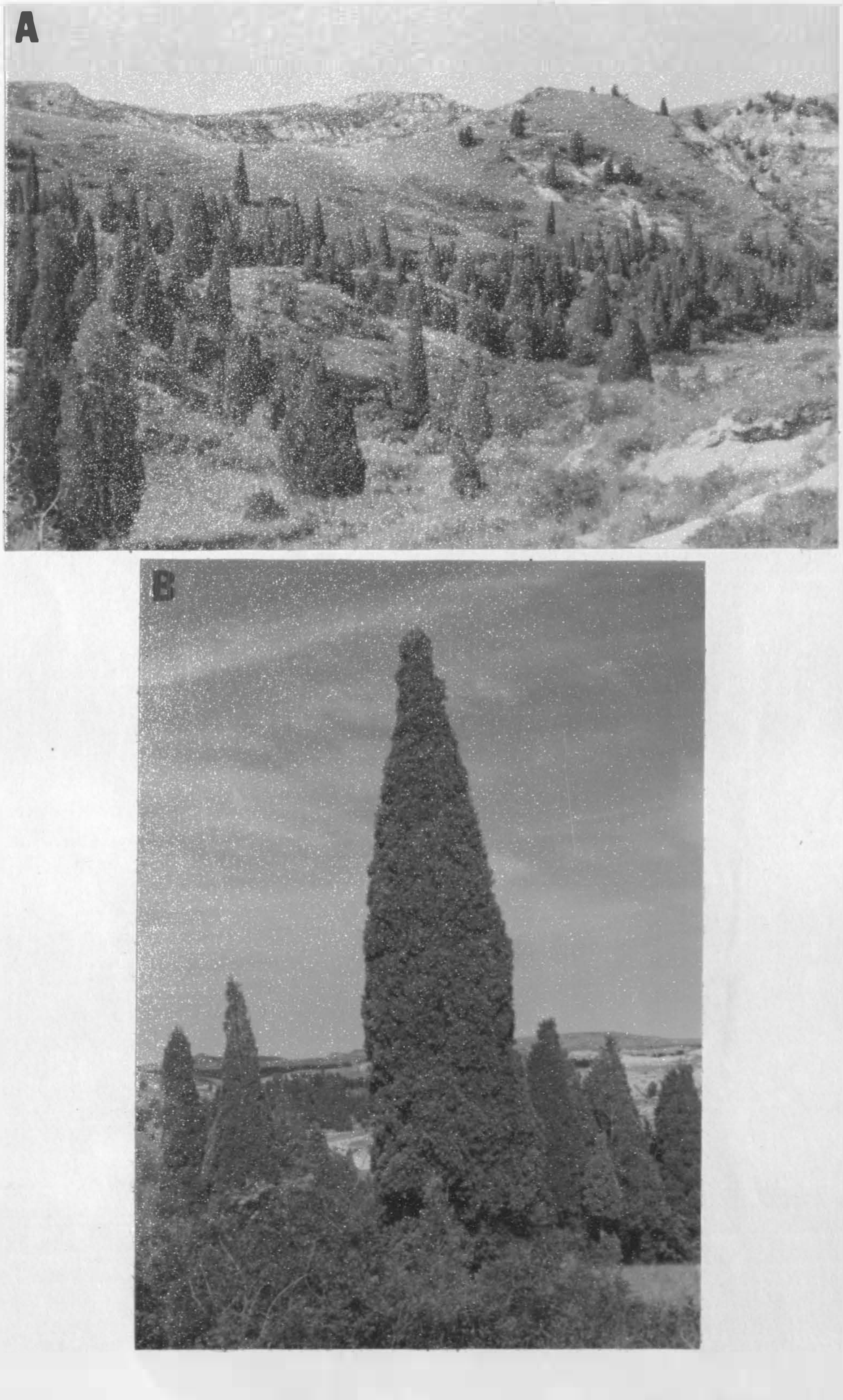

A
B

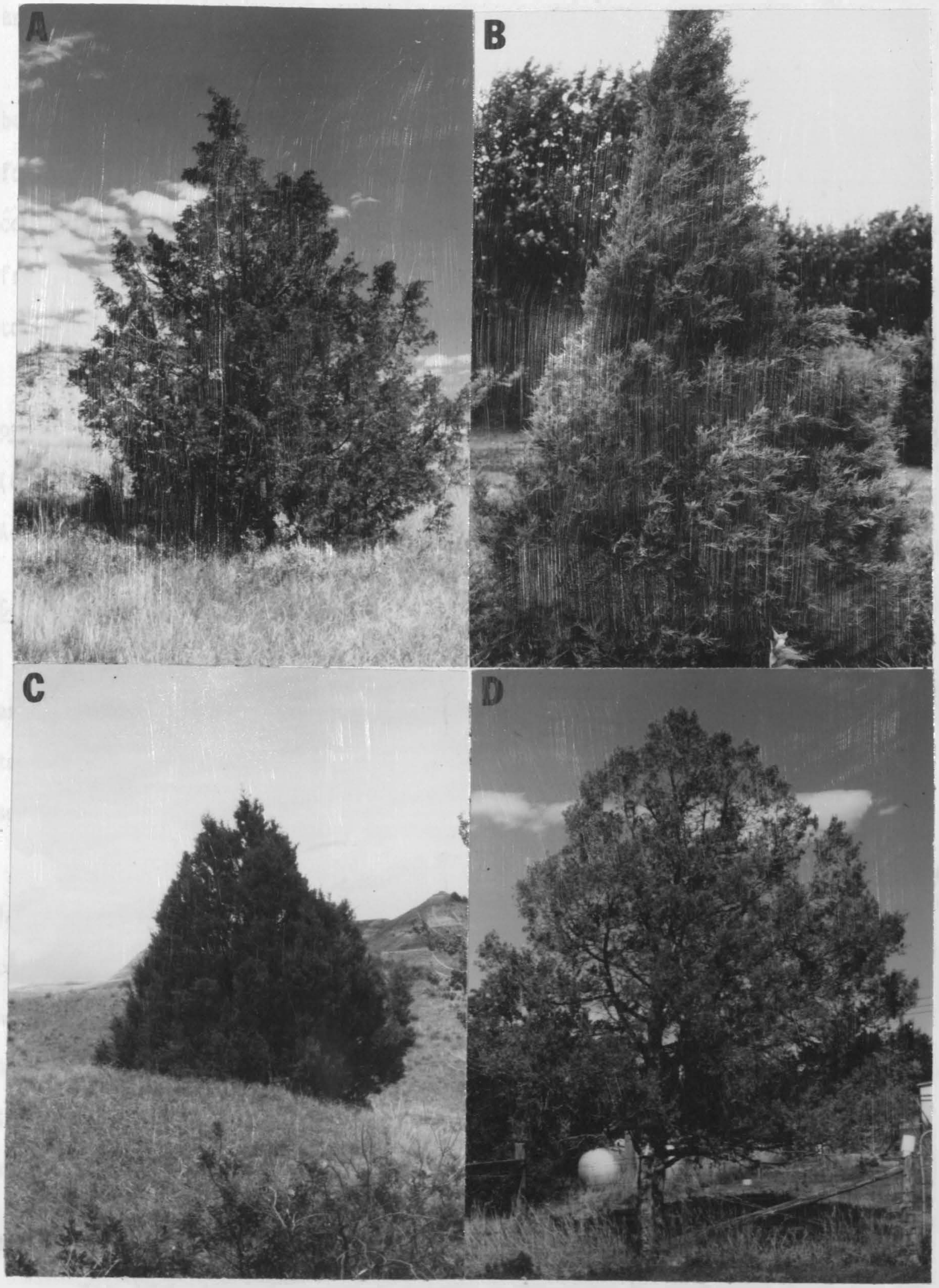
Figure 5. Various forms of *Juniperus scopulorum* found in Western North Dakota.

A. A typical *J. scopulorum*. Note the many branches and rounded form.

B. A pyrimidal juniper (columnar form on the upper portion of the tree) near the burning lignite vein in Billings County.

C. A juniper approximately 2 miles from the burning lignite vein in Slope County which shows only a slight variation from the typical form. Note the thickness of the outer leaves as compared to the juniper above (A).

D. A "columnar" juniper that was transplanted to the Logging-Camp Ranch in the 1920's and has reverted back to a more rounded form.


A
B
C
D

and C).

If a columnar juniper is transplanted to a site away from the burning lignite it will normally revert back to the true *J. scopulorum* form within 2-5 years (Staudinger 1966). Several years ago four columnar junipers were transplanted on the Logging Camp Ranch (3-4 miles from the burning lignite vein) and all of these trees have reverted back to the form of *J. scopulorum* (Figure 5D).

During the course of writing this report, an additional report of pyrimidal junipers in association with a burning coal deposit was found (Cringen and Dix 1975). These junipers are located in the Sheridan-Acme-Decker coal basin, north of Sheridan in Sheridan County, Wyoming.

Gas Chromatography

The samples of fumes from Slope County and the laboratory were analyzed for ethylene by gas chromatography. The unknowns were compared to the standards on the basis of peak heights and the results are summarized in Table 1.

Ethylene was found in considerable amount in all samples from the laboratory burning. Of the samples from Slope County, only those collected directly from the vent holes contained enough ethylene to be measured by the gas chromatogram. Of the four vent holes sampled, number 1 had the highest ground level temperature and the lowest concentration of ethylene. In sample vent number 2 the opposite condition was observed.

Bioassays Conducted on Site

The bioassays conducted in Slope County yielded very little

Table 1. Concentration of Ethylene in Fumes from Burning Lignite as Analyzed by G. C. All Values from Slope County are Averages of Three Replications.

Source	Vent No.	Concentration (ppm)	Remarks
Slope County	1	5.0	highest vent temp.
	2	52.5	lowest vent temp.
	3	14.4	
	4	9.4	
	Ave.	20.3	
	Sample No.		
Simulated burning in laboratory	1	3260	collected after heating lignite for 1 minute
	2	4960	
	3	4130	
	Ave.	4116.6	

information. Large diurnal temperature fluctuations and strong winds greatly reduced the value of the peas and tomatoes as test plants. Of the three characteristic responses of pea to ethylene, the peas exhibited only a slight geotropic distortion. There were no differences noted in length or diameter between the control and the treated seedlings. The tomatoes were almost completely destroyed by the wind and therefore the tomato bioassay was discontinued after several hours.

Bioassays Conducted in Laboratory

Bioassays for Ethylene

When the etiolated pea bioassay was carried out under the controlled conditions of the laboratory, the characteristic "triple-response" to ethylene was evident to some extent on all of the peas used in this test except the controls. Figure 6 illustrates the response of etiolated peas to ethylene, and Figure 7 illustrates the response to the lignite fumes. The amount of inhibition of elongation was used as an indication of the amount of ethylene present. The results are summarized in Figure 8. The amount of ethylene in lignite fumes was calculated from the graph to be approximately 4,950 ppm (average of all treatments).

The tomato petiole bioassay was also found to work satisfactorily in the laboratory. The change in the petiole-stem axis angle during the treatment period was used as an indication of the amount of ethylene present. The photographs in Figure 9 illustrate the effect of ethylene on tomato petiole-stem axis angles. The graph in Figure 10 summarizes the results of this bioassay. From this, the amount of ethylene in lignite fumes was calculated to be approximately 4,300 ppm (average of all treatments).

The gas chromatography, the etiolated pea bioassay, and the tomato petiole bioassay all yielded similar results. There were some differences in the amount of ethylene calculated to be in the burning lignite fumes. This could be attributed to compounds other than ethylene (i.e. acetylene) in the lignite fumes which have different additive or synergistic effects in the two bioassays and no effect in the gas

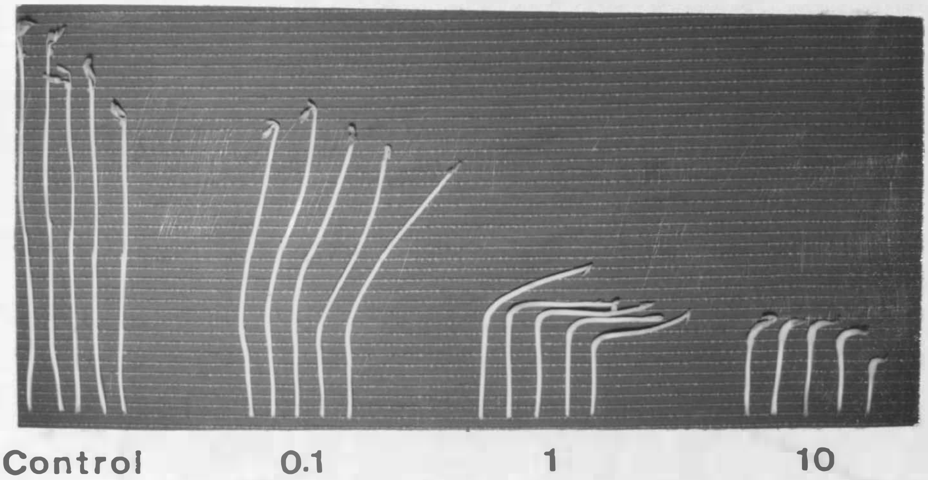


Figure 6. Effect of ethylene on the growth of etiolated pea seedlings. Values under each group of seedlings represents the concentration (ppm) of ethylene applied for 72 hours (graphically shown in Figure 8).

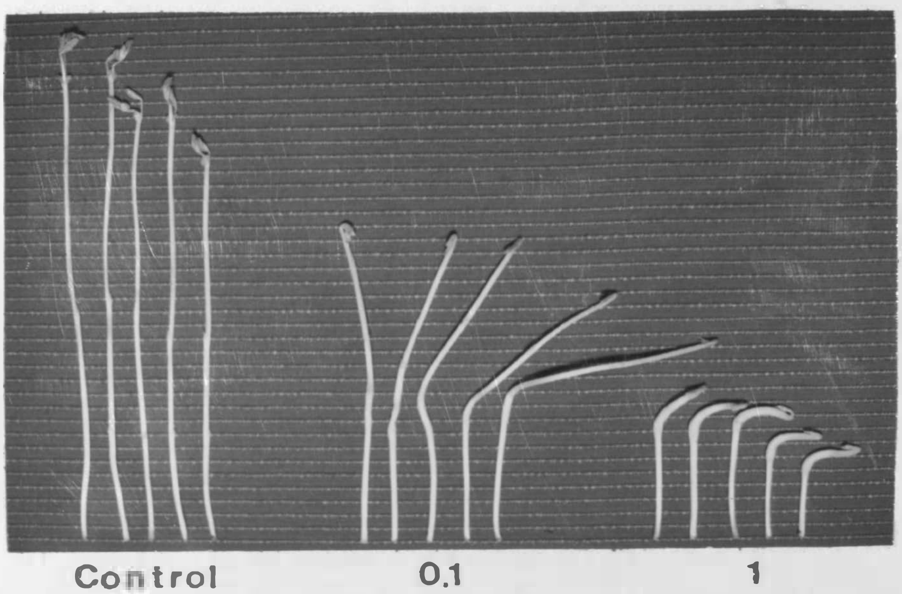


Figure 7. Effect of burning lignite fumes on the growth of etiolated pea seedlings. Values under each group of seedlings represents the concentration (ml/L) of lignite fumes applied for 72 hours. The concentration of ethylene in the lignite fumes, as determined from Figure 8, was found to be 4,950 ppm.

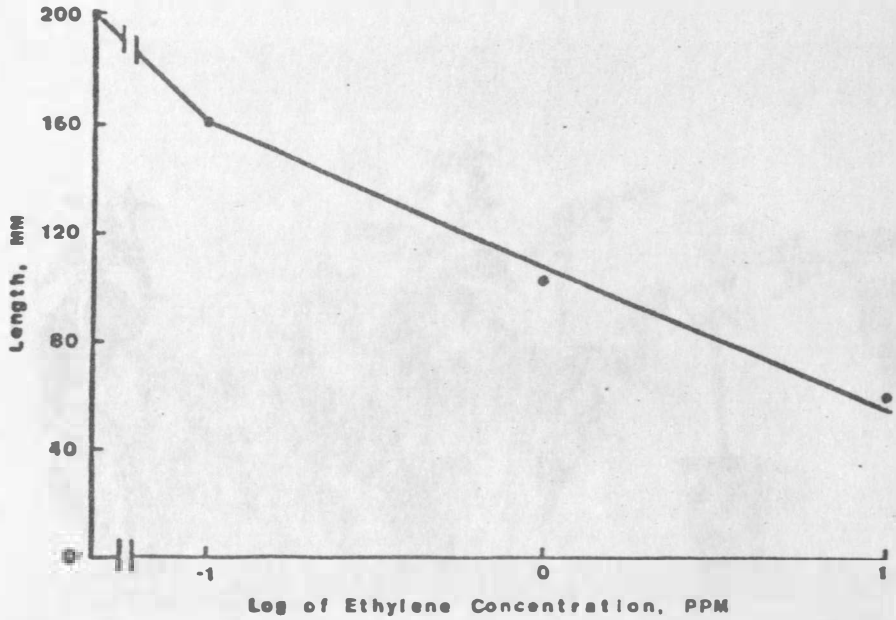


Figure 8. Elongation of the epicotyls of intact etiolated pea seedlings treated for 72 hours with various concentrations of ethylene. The concentration of ethylene is the concentration inside the bioassay bag. To find the concentration of ethylene in the lignite fumes, multiply the results from the graph by the dilution factor of the treatment in question. All of the epicotyls were approximately 40 mm long at the beginning of the treatment.

Figure 9. Ethylene-induced epinasty of tomato petioles after being treated for 24 hours with the following: top (left to right) 1 ppm ethylene, 0.5 ppm ethylene, 0.1 ppm ethylene; bottom (left to right) 1 ppm ethylene, control, 0.1 ml/l burning lignite fumes. The concentration of ethylene in the lignite fumes, as determined from Figure 10, was found to be 4,300 ppm.

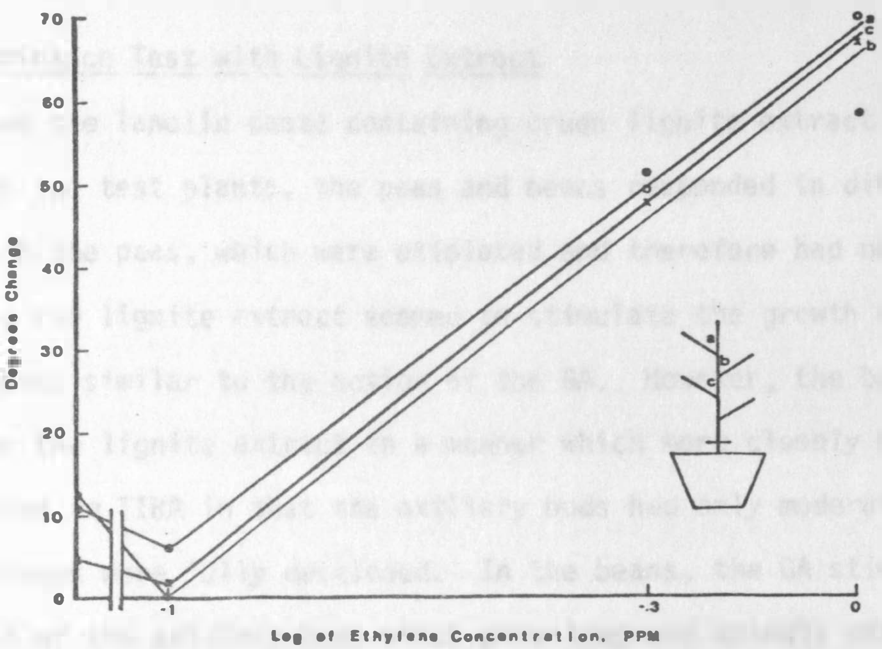


Figure 10. Changes in tomato petiole-stem axis angles when treated for 24 hours with various concentrations of ethylene. Three petiole-stem axis angles were measured on each plant (a, b, and c on the insert) and each line represents one of these angles. The concentration of ethylene is the concentration inside the bioassay bag. To find the concentration of ethylene in lignite fumes, multiply the results from the graph by the dilution factor of the treatment in question.

chromatographic analysis.

Apical Dominance Test with Lignite Extract

When the lanolin paste containing crude lignite extract was applied to the test plants, the peas and beans responded in different ways. With the peas, which were etiolated and therefore had no leaf expansion, the lignite extract seemed to stimulate the growth of the axillary buds similar to the action of the GA. However, the beans reacted to the lignite extract in a manner which more closely resembled the reaction to TIBA in that the axillary buds had only moderate growth and the leaves were fully developed. In the beans, the GA stimulated the growth of the axillary buds which grew long and spindly with very small leaves. Additional work is required before definite results can be drawn.

Cucumber Root Growth Test with Lignite Extracts

The results of the cucumber root growth test are summarized in Figure 11. The extracts were found to have little effect on the growth of cucumber roots when applied at the lower concentrations. However, with the high concentration (10,000 ppm) the root growth of cucumber seedlings was severely inhibited and a swelling of the tissue at the basal end of the hypocotyl was observed.

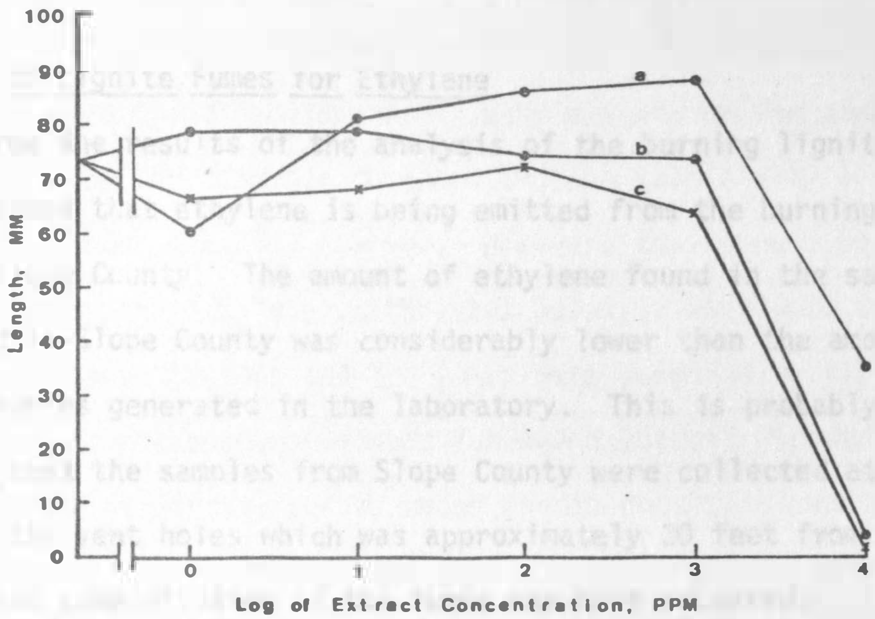


Figure 11. Growth of intact cucumber roots that were germinated and grown for 5 days in various concentrations of 3 lignite extracts. The humic acid, fulvic acid, and crude lignite extracts are represented by lines a, b, and c respectively.

DISCUSSION

Analysis of Lignite Fumes for Ethylene

From the results of the analysis of the burning lignite fumes it is evident that ethylene is being emitted from the burning lignite vein in Slope County. The amount of ethylene found in the samples collected in Slope County was considerably lower than the amount found in the samples generated in the laboratory. This is probably due to the fact that the samples from Slope County were collected at ground level in the vent holes which was approximately 30 feet from the actual burning and some dilution of the fumes may have occurred.

Once the fumes have escaped from the vent holes and have mixed with the surrounding air, the ethylene has become too dilute for detection by the methods utilized in this study. Therefore, the amount of ethylene that is actually reaching the columnar junipers was not determined.

Gas Chromatography

The minimum amount of ethylene detectable with the chromatographic technique used was about 1 ppm. The sensitivity of the gas chromatogram could be increased, by the use of a technique described by Bellar *et al* (1962), to detect concentrations as low as 1 ppb or less. By the use of a more sensitive technique, coupled with prior concentration of the sample by passing large quantities of air through an ethylene trap (Young *et al* 1948, Stephens and Burleson 1967), it may be possible to measure the minute quantities of ethylene found in the air surrounding the columnar junipers.

Bioassays for Ethylene

The etiolated pea bioassay and the tomato petiole bioassay proved to be unsuitable for detection of the ethylene at the low concentrations found near the columnar junipers and the sensitivity of these bioassays can not be easily changed. However, they should be sufficient for quantifying the ethylene at the vent holes if they were carried out in an enclosed chamber and the fumes were pumped directly from the vent holes into the chamber without further dilution. Growth of pea and tomato planted at various points under garden conditions would also be a valuable test of ethylene in the area.

Lignite Extract Tests

Both the cucumber root growth test and the apical dominance test proved to be inconclusive for testing the crude lignite extract. It was shown that the extract did have an effect on plant growth; however, the tests were inadequate for demonstrating what type of growth regulator was present.

Numerous companies and individuals, such as Minnesota Peat Cellulose and Chemical Corporation (Chisholm, Minnesota) and John Willard (Department of Chemistry, South Dakota School of Mines and Technology, Rapid City, South Dakota), have tried to capitalize on the commercial properties of humic substance from lignite and other sources as soil ammendments or plant growth stimulators. Therefore, many investigators have been working on the problem of identifying plant growth regulators in humic substances (Schnitzer and Khan 1972). The various methods used by these investigators for extraction, fractionation, and characterization have led to many diverse conclusions as to the nature of this material (Fowkes 1975, Wershaw and Pickney 1977, and Grant 1977). The structure and physiological properties of humic substances are still relatively unknown and much more research needs to be done in this area.

Soil Related Factors

There are a couple of factors which diminish the probability of the columnar form resulting from a soil factor. The first is that the only place in the North Dakota Badlands where *J. scopulorum* is found growing in the columnar form is near a burning lignite vein. If there is a morphogenetic compound in the soil which is causing the columnar growth, then one would expect to find columnar junipers throughout the soils of the coal bearing strata.

The second factor is the fact that true *J. scopulorum* grew at the columnar juniper sites until the fires began at the respective sites. The presence of old true *J. scopulorum* (Slope Co.) and other intermediate forms (Billings Co.) intermingled with the columnar junipers illustrates this point. Staudinger (1966) estimated, by annual growth ring counts, that the old true *J. scopulorum* at the Slope County site were all over 100 years old and their basic growth form was probably established prior to the start of the fire. The true *J. scopulorum* which were growing at the Billings County site at the start of the fire there in 1951, have since taken on columnar form, giving rise to various intermediate forms (Staudinger 1966). The recent report of the pyrimidal junipers in association with a burning coal deposit in Wyoming (Cringen and Dix 1975) also supports the idea that as soon as the coal starts burning, young junipers change first to pyrimidal and then a columnar form.

There is the possibility that the columnar form could result from mutagenic substance released as a product of combustion of the lignite. Mutagenic hydrocarbons, i.e. 3,4-benzopyrene, are readily formed from the pyrolysis of almost any organic material, including coal (Arcos 1978). The vast majority of the research on these compounds utilize animal species as the test subjects and therefore very little is known of the effects of these compounds on plants. However it does not appear that the columnar junipers are the result of a mutation, since when they are transplanted away from the burning lignite they normally revert to the rounded form.

Ethylene

The evidence presented in this paper supports the hypothesis that ethylene is the causal agent of the columnar growth of the *J. scopulorum* growing in the vicinity of the burning lignite vein. Although the long term effects of exogenous ethylene on tree species have not been extensively studied, it has been shown that ethylene does have an effect on many trees (Brown and Leopold 1972, de Wilde 1971) including juniper species (Heck and Pires 1962) at very low concentrations. Although it would take a more sophisticated technique to accurately quantify the amount of ethylene in the atmosphere around the columnar junipers, it was shown that a substantial amount of ethylene is being emitted from the burning lignite vein. More definite proof could be provided by treating *J. scopulorum* with ethylene to see if a columnar juniper results. Such an experiment would take several years to complete and is therefore beyond the scope of this study.

Indications are that the junipers which receive the most ethylene (i.e. closer to the source) are the most columnar. It has been shown previously (Sandberg *et al* 1975) that the conditions of burning can influence the quantity and composition of the effluent gases. The depth of the overburden, the moisture content of the lignite, and the amount of ventilation which the fire receives could all influence the amount of ethylene released. Also, since most of the junipers are growing in the valleys, there is a good probability of a temperature inversion forming which would enable the ethylene concentration to build up. Therefore, it is conceivable that the concentration of ethylene surrounding the columnar junipers could vary considerably from what it was when it was sampled for this study.

The ethylene that is reaching the columnar junipers does not elicit a typical reported ethylene induced response. The columnar junipers appear to fit the definition of a phenocopy (Serra 1966) in that the phenotype, but not the genotype, of the *J. scopulorum* has been altered by the environment to resemble the phenotype of genetically columnar junipers. That is to say the environment is altering the expression of the genetic material and not the genetic material itself. The fact that when columnar junipers are transplanted away from the burning lignite they normally revert to the true or rounded form would lend support to this concept.

Staudinger (1966) reported that only one of approximately 40 columnar junipers transplanted to the North Dakota State University campus at Fargo has remained columnar. This would be consistent with the phenocopy concept since with some individuals the phenocopy agent may react to such a degree that the phenocopy may become fixed as a mutant (Serra 1966).

It would be possible, by vegetative propagation, to determine if the genetic material had been altered. Dale Herman (personal communication) propagated about 25 columnar selections by rooting cuttings and none of them are maintaining their columnar form. This indicates that the genetic material had not been altered; however, further studies in this area could be carried out. Cloning of the various stages of the columnar form by tissue culture would provide much information on this point.

SUMMARY AND CONCLUSION

In a previous study it was concluded that the columnar junipers growing in the vicinity of burning lignite deposits were an ecologically-induced variant of the *J. scopulorum* with the phenotype requiring a certain environment for expression. A search of the literature and a study of the environment in this area was undertaken to determine the possible morphogenetic factors that could lead to a change in the growth habit of the junipers.

It was considered that the columnar form could result from a morphogenetic substance in the soil. Although there are weak morphogenetic substances in the soil that could be derived from lignite, these substances should be found throughout the soils of lignite bearing strata. In addition, the presence of old *J. scopulorum* intermingled with the columnar junipers, coupled with the fact that the only place the columnar junipers have been found is in association with a burning lignite deposit, diminishes the probability of the columnar form resulting from a soil factor.

Of the constituents of the burning lignite fumes, ethylene was the main morphogenetic substance found. Ethylene is known to induce various responses in plants at very low concentration and it was found that a substantial amount of ethylene was being emitted from the burning lignite. These results strongly implicate ethylene as being the primary cause of columnarity in the junipers; however, synergistic effects are probable with the other gases found in the fumes.

More definite proof could be provided by testing the long term effects of ethylene on *J. scopulorum* and the characterization of the morphogenetic compounds found in soil or coal derived humic substances. Also, much could be learned about the mechanism(s) involved in the change to the columnar form, by cloning of the columnar and the various intermediate forms found in the area.

LITERATURE CITED

Abeles, F. B. 1973. Ethylene in plant biology. Academic Press. New York.

Arcos, J. C. 1978. Cancer: Chemical factors in the environment -- an overview, Part I. American Laboratory. 10(6): 65-73.

Bellar, T., J. E. Sigsby, C. A. Clemons, and A. P. Altshuller. 1962. Direct application of gas chromatography to atmospheric pollutants. Analytical Chemistry. 34:763-765.

Brown, K. M. and A. C. Leopold. 1973. Ethylene and the regulation of growth in pine. Canadian Journal of Forest Research. 3:143-145.

Cringen, A. T. and R. L. Dix. 1975. Ecological theme analysis and inventory of potential landmarks of the great plains natural region. Report to the National Park Service, U. S. Dept. of Interior, contract no. 14-10-9-900-248, p. 483.

Crocker, W. 1948. Growth of Plants. Reinhold Publishing Corp. New York. pp. 139-203.

de Wilde, R. C. 1971. Practical applications of (2-chloroethyl) phosphonic acid in agriculture production. Hortscience. 6:364-370.

Fassett, N. C. 1944. *Juniperus virginiana*, *J. horizontalis*, and *J. scopulorum* - I. The specific characters. Bulletin of the Torrey Botanical Club. 71:410-418.

Fassett, N. C. 1945. *Juniperus virginiana*, *J. horizontalis*, and *J. scopulorum* - V. Taxonomic treatment. Bulletin of the Torrey Botanical Club. 72:480-482.

Forsyth, W. G. C. 1947. Studies on the more soluble complexes of soil organic matter - I. A method of fractionation. Biochemical Journal. 41:176-181.

Fowkes, W. W. 1975. Some characteristics of natural and coal-derived humates: with bibliography. Grand Forks Energy Research Center IC-75/1. 66 pp.

Freeman, P. G. and W. W. Fowkes. 1968. Coal-derived humus: plant growth effects. Bureau of Mines Report of Investigation 7203. 16 pp.

Grant, D. 1977. Chemical structure of humic substances. Nature. 270: 709-710.

Heck, W. W. and E. G. Pires. 1962. Effects of ethylene on horticultural and agronomic plants. Texas Agricultural Experiment Station M. P. 613. 12 pp.

Herman, D. E. 1976. North Dakota State University. Fargo, N. D. Personal communication.

Hurlbut, C. S. Jr. 1956. Dana's Manual of Minerology. John Wiley and Sons, Inc. New York. pp. 133-149.

Jacobs, M. R. 1954. The effect of wind sway on the form and development of *Pinus radiata* D. Don. Australian Journal of Botany. 2:35-51.

Landers, W. S., J. B. Goodman, and D. J. Donaven. 1961. Low-temperature carbonization assays of coals and relation of yields to analyses. Bureau of Mines Report of Investigation 5904. 41 pp.

Leopold, A. C., K. M. Brown, and F. H. Emerson. 1972. Ethylene in the wood of stressed trees. Hortscience. 7:175.

Linehan, D. J. 1976. Some effects of a fulvic acid component of soil organic matter on the growth of cultured excised tomato roots. Soil Biology and Biochemistry. 8:511-517.

Machlis, L. and J. Torrey. 1956. Plants in Action. W. H. Freeman and Company. San Francisco. p. 29.

McCallan, S. E. A., A. Hartzell, and F. Wilcoxon. 1936. Hydrogen sulphide injury to plants. Contributions from Boyce Thompson Institute. 8:189-197.

Mitchell, J. W. and G. A. Livingston. 1968. Methods of studying plant hormones and growth-regulating substances. Agriculture Handbook no. 336. U. S. Government Printing Office. Washington, D. C. pp. 74-75.

Neel, P. L. and R. W. Harris. 1971. Motion-induced inhibition of elongation and induction of dormancy in liquidambar. Science. 173: 58-59.

Noggle, G. R. and G. J. Fritz. 1976. Introductory Plant Physiology. Prentice-Hall Inc. Englewood Cliffs, N. J . pp. 482-485.

Robitaille, H. A. and A. C. Leopold. 1974. Ethylene and the regulation of apple stem growth under stress. Physiol. Plant. 32:301-304.

Ross, C. W. 1974. Plant Physiology Laboratory Manual. Wadsworth Publishing Company Inc. Belmont, California. pp. 143-144.

Sandberg, D. V., S. G. Pickford, and E. F. Darley. 1975. Emissions from slash burning and the influence of flame retardant chemicals. Journal of Air Pollution Control Association. 25:278-281.

Schnitzer, M. and S. U. Khan. 1972. Humic substances in the environment. Marcel Dekker, Inc. New York.

Serra, J. 1966. Modern Genetics, vol. 2. Academic Press. New York. pp. 273-332.

Staudinger, J. D. 1966. A chromatographic and ecological study of the columnar juniper of Western North Dakota. Masters Thesis, University of North Dakota. Grand Forks. 41 pp.

Stephens, E. R. and F. R. Burleson. 1967. Analysis of the atmosphere for light hydrocarbons. Journal of Air Pollution Control Association. 17:147-153.

Treshow, M. 1970. Environment and Plant Response. McGraw-Hill Book Company. New York. pp. 245-249, 254-369.

U. S. Forest Service. Burning Coal Vein - Custer National Forest. U. S. Forest Service/Northern Region, Custer National Forest. Billings, Montana.

Weaver, R. J. 1972. Plant Growth Substances in Agriculture. W. H. Freeman and Company. San Francisco. pp. 83-89.

Wershaw, R. L. and D. J. Pickney. 1978. Methylation of humic acid fractions. Science. 199:906-907.

Young, R. E., H. K. Pratt, and J. B. Biale. 1948. Micromanometric estimation of ethylene in the emanations of ripening fruit. American Journal of Botany. 35:814.